FISHER MOLECULAR BIOLOGY

GOTAQ FLEXI DNA POLYMERASE GREEN G2 FS-T-0531

Description

GoTaq Flexi DNA Polymerase Green G2 is new generation of Taq polymerase that gives robust amplification and high DNA yield in shorter PCR running time (15-30 s/kb extension). GoTaq Flexi DNA polymerase supports the robust and reliable amplification of a wide range of DNA templates up to 6 kb. GoTaq Flexi is provided with 5× Green Reaction Buffer allowing reactions to be loaded directly into gels without the extra adding of loading dye and with a 10x Colorless Buffer. GoTaq Flexi lacks 3' 5'exonuclease activity. Resulting PCR products have an A-overhang and suitable for cloning.

Description	FS-T-0531
GOTAQ Flexi DNA Polymerase Green G2	500 U
5X Green Reaction Buffer	2 x 2 ml
10 X Colorless Buffer	4X1 ml
50 mM MgCl2 Solution	2X1 ml

Storage Buffer

20mM Tris-HCl, 1mM dithiothreitol, 0.1mM EDTA, 100mM NaCl, Stabilizer, 50% glycerol, pH 8.0 (25°C).

5X Green Reaction Buffer

The 5X Green Reaction Buffer contains 2 dyes (blue & yellow) that separate during electrophoresis to monitor migration progress. The blue dye migrates at the same rate as a 3-5kb DNA fragment in a1% agarose gel,the yellow dye migrates a t a rate faster than primers (<50kb) in a 1% agarose gel.

10X Colorless Buffer

The Colorless Buffer is used when direct fluorescence or absorbance readings are required without prior purification of the amplified DNA from the PCR.
Proprietary formulation supplied at pH8.5 containing Tris-HCl, KCl and PCR enhancers and do not contain Mg.

Unit Definition

1 unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid-insoluble material in 30 minutes at 74°C.

Storage Conditions

Store all components at -20ºC in a non-frost-free freezer.

Quality Control

No endonuclease activity, nicking activity, exonuclease activity, or priming activity has been detected.

Genomic DNA contamination:

The product must be free of any detectable DNA contamination as evaluated through PCR. Thus, it is suitable for the amplification of bacterial and fungal DNA based on 16S and 18S rRNA PCR assays.

Nuclease assays

0.2-0.3 µg of pNZY28 plasmid DNA are incubated with 5 U of GoFlexi Taq DNA polymerase, in 1× Reaction Buffer, for 14-16 hours at 37 °C. Following incubation, the DNA is visualised on a Green Gel Safe -stained agarose gel. There must be no visible nicking or cutting of the nucleic acid. Similar tests are performed with buffer and MgCl2 solution.

Functional assay

GoFlexi Taq DNA polymerase is extensively tested for performance in a polymerase chain reaction (PCR) of different-sized DNA fragments (1 and 2.5 kb) from human genomic DNA in the presence of 5× Green Reaction Buffer and MgCl2 solution. The resulting PCR products are visualized as single bands in a Green Gel Safe stained agarose gel

Recommended Protocol

On ice, prepare each of following master mixes, combine, and place in heated (to 95°C) thermal cycler:

PCR using Green reaction buffer

For 50µl PCR Reaction	Volume	Final Conc.
GOTAQ Flexi DNA Polymerase Green	0.25-1 µl	1.25 U
5X Green Reaction Buffer	10 µl	1 X
50 mM MgCl2 Solution	2.5mM	(1.5~4.0) mM
dNTP mix (2.5 mM each)	0.4 µl	(0.25-0.5)
Template DNA **(see below)**	5 pg-0.5ug	0.1-0.5 uM
Forward Primer	0.25 ul	0.1~0.5 µM
Nuclease free water	up to 50 µl	

PCR Reaction using Colorless buffer

For 50µl PCR Reaction	Volume	Final Conc.
GOTAQ Flexi DNA Polymerase G2	0.25-1 µl	1.25 U
10X Colorless Buffer	5 µl	1 X
50 mM MgCl2 Solution	2.5mM	(1.5~4.0) mM
dNTP mix (2.5 mM each)	0.4 µl	(0.25-0.5)
Template DNA **(see below)**	5 pg-0.5ug	0.1-0.5 uM
Forward Primer	0.25 ul	0.1~0.5 µM
Nuclease free water	up to 50 µl	

General Cycling Conditions :

Step	Temp (°C)	Time	Cycle
Initial Denaturation	95	3 min.	1
Denature	94	30 sec.(**)	25 ~ 35
Anneal	50~65(*)	30 sec.	
Extend	72	15 ~ 30 sec./kb**	
Final Extension	72	5 min.	1

* Annealing temperature should be optimized for each primer set based on the primer Tm; typically, it should be Tm- 5 °C. ** For DNA fragments higher than 3 kb to 6 kb in size, it may be beneficial to use 20 sec for denaturation and 30-60 sec/kb for extension

Primer Design

PCR primers generally range in length from 15–30 bases and are designed to flank the region of interest. Primers should contain 40–60% GC, and care should be taken to avoid sequences that might produce internal secondary structure. The 3'-ends of the primers should not be complementary to avoid the production of primer- dimers. Primer-dimers unnecessarily remove primers from the reaction and result in an unwanted polymerase reaction that competes with the desired reaction. Avoid three G or C nucleotides in a row near the 3'-end of the primer, as this may result in non- specific primer annealing. Ideally, both primers should have nearly identical melting temperatures (Tm), allowing their annealing with the denatured template DNA at roughly the same temp.

DNA template

The optimal amount of starting material may vary depending on its quality and complexity. In general, we recommend using 10 ng to 500 ng of genomic DNA templates, although the enzyme is sensitive enough to amplify fragments from as little as 5 pg of human gDNA,for example. Lower amounts of template may be used for amplification of less complex DNA (typically 1-20 ng). When using a cDNA synthesis reaction as template do not exceed 10% of the final PCR reaction volume.

Applications:

- Routine PCR
- Genotyping
- Library construction
- TA Cloning
- Primary Extension
- Colony PCR – Multiplex PCR

Troubleshooting

No product amplification or low yield

A) Inadequate annealing temperature
The reaction mix composition may affect the melting properties of primers and DNA. Adjust the annealing temperature to accommodate the primer with the lowest melting temperature (5 °C to 10° C lower).

B) Presence of PCR inhibitors
Some **DNA** isolation procedures, particularly genomic **DNA** isolation,can result in the co-purification of PCR inhibitors. Reduce the volume of template DNA in reaction or dilute template DNA prior to adding to the reaction. Diluting samples even 1: 10,000 has been shown to be effective in improving results, depending on initial **DNA** concentration.

Presence of no-specific bands
C) Non-specific annealing of primers
Adjust annealing conditions and/or design another set of primers, by increasing the length and avoiding complementary sequences.

D) Mg2+ concentration is too high
Generally, 2-3 mM MgCl2, typically 2.5 mM final concentration, works well for most of PCR reactions. Optimal concentration depends on target template, buffer and dNTPs.
Optimize magnesium concentration by supplementing MgCl2 in 0.5 increments up to 4 mM.

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36 Terry Drive
Trevose, PA 19048 - USA